

FIG. 1B

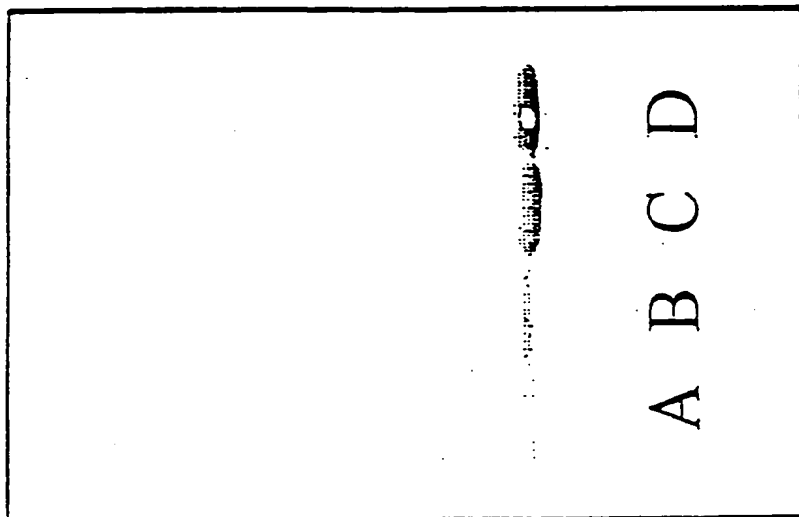
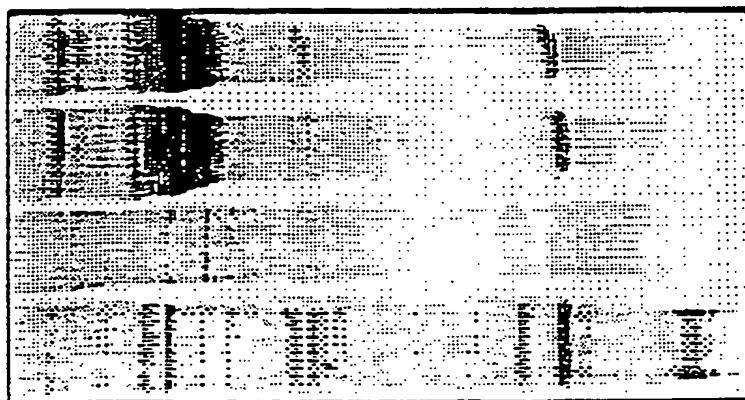


FIG. 1A



A B C D

FIG. 2

1 AAACCAAGCAGGACACAGCAGCCATGAAGGCCCTTCGTTGCAGTCGCCCTTTTGTCTGCA 60
M K A F V A V A L L S A

61 GTTCCCGTGGCACATGCTGCCCTCAAGACTGACGTAGCCAGTGGACCTGCCCGGTTCCTGGT 120
V S V A H A A L K T D V A S G P A G S G 2/19

121 GCACTAAGTCTAGGAGTTGGAGGCTTCCCGTCCGGTGCTTCGCTTGGCAGCCCTTAGTGCC 180
A L S L G V G G F P S G A S L G S L S G

181 GTAACCCCTCTCTGGTGCTGGCTCTTCCGTGTCTGGCCGCCCTGGATCCCCTGGATCGGCT 240
V T L S G A G S S V S G R P G S P G S A

241 GGTCCCTAGCTCTGGACCCGCAGTGTCG 267
G P S S G P A V S

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FIG. 3

1 CGGACGCACACTCCTGCAGGAAGTCACTAGTTCGCCCAACATGAAGCTGCTGTGCA 60
M K L L C A

61 CTAGCCCTCGTTGCCCTTGGA CTTCGGCAGCGCTTACCTTGGTGGCTTCGGCGGC 120
L A L V A L G L P F G S A Y L G G F G G

121 CTCGGTGGTTGGGGTGGCGGTCTCGGTGCCATCTTTGGCCCCAGGAGCTTATCCCGGTTTC 180
L G G W G G G L G A I F G P G A Y P G F

181 TATGGCCTTAACAGCGTGCACCTCTTGGGCGGCAGGTTCCACCATCTCTTCGGGCGATTTC 240
Y G L N S V H L L G G R F H L F G R F

241 CCGCCACCACCGGTATTGGAGCTGCTGAAGCGCAGGGGAACCTAAGCCCATACCCCTCTT 300
P P P G I G A A E A Q G N L S P Y P L

301 GACATCAACACCGTCCAAGACCCGAACTGGCCACCCCATGGTACGCGTTGTCTACGGCGG 360
D I N T V Q D D P N W P P H G T R C L R R

361 AGTCTTGGGGAGCGCCTCTGACCCTGACCAGTCCCAATTCACAGGATGTGCCTGTCCC 420
S L A G A P L T L T S P N S T G C A C P

FIG. 3(CONTD.)

FIG. 4

1 GATCGGCACGAGGTCAAGGGAGCCCTCCTTCAGCAACAACAAGCATCGCAGGTTAAGGGA 60
D R H E V K G A L L Q Q Q Q A S Q V K G

61 GCCCTCAAGGGAGCAATCAAGGGTGGTCTTCTTCAGCAACAAGCCCAATCCCAGTCCAA 120
A L K G A I K G G L L Q Q Q A Q S Q V Q

121 GGAGCTCTTAAGGGAGCCGTCAAGGGAGCCCTCCTTCAGCAACAACAGGCATCACAGGTC 180
G A L K G A V K G A L L Q Q Q Q A S Q V

181 AAGGGAGCCCTCAAGGGAGCCATCAAGGTCTGTCTCCTTCATCAGCAAGCCCAATCCCAA 240
K G A L K G A I K V C L L H Q Q A Q S Q

241 TCCCAAGTTCAGGGAGCTCTTAAGGGAGCTG 271
S Q V Q G A L K G A

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FIG. 5

1 GGAAGTAGCGAGCATCCGCACTGGGGTCTTTTGGCTGCATTGCTTTTCTTCTTCAGC 60
E V A S I R T G V F L A A F A F L L S A

61 GATCCATAACAATGGCCAGTCAIGTAGATGCAGCCCCACTCGACGTCCTATGCCATC 120
I H N N G Q S C V D A A P T R R P M P S

121 TCCTCCTGGATGTGCTGGTCCCTGGCTGTTTACTGGTATTGCTACTCTTCTAAGACCTGG 180
P P G C A G P G C F T G I A T L L R P G

181 TCAAGGACAGCAACCTGGTCAAGGACAGCAACCTGGTCAAGGGCGTCTCCAATGCCACG 240
Q G Q Q P G Q G Q Q P G Q G R P P M P R

241 TCCAGGACCTGTTCAGGACATCTGGATCACCTCAAGGAAGACCCCAATGGAGCACCTCG 300
P G P V P G T S G S P Q G R P N G A P R

301 TCCAGGACCTGTTCCTGGAAACATCTGGATCACCTCAAGGAAGACCTAAGCAAGACCTCG 360
P G P V P G T S G S P Q G R P N A R P R

361 TCCAGGACCTGTTCCTGGACACCAACTGTATCCTCTCCCGGATCATCTCTGGGTCATC 420
P G P V P G T P T V S S P G S S P G S S

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421 TCCAGGAATATCTTAGGAACGCCCTCTAGGAACACCTCTAGGAACACCTCAAGGATCACC 480
P G I S L G T P L G T P L G T P Q G S P

481 TTTTGGATCATCTTTGGATCATCGATAGGATCACCTCCTGCAACATCTCCTGGATCATC 540
F G S S L G S S I G S P P A T S P G S S

541 TTCTCCGTCACCTCCTGGATCAGCGAATGTGAACCTGCTGGGTCTCGACCAATTCCGCGG 600
S P S P P G S A N V N L L G P R P I R G

601 TCCTGGAAGGCATTGACGGGACCAGTTCTGCTGTGTATTCTCCTCCGTGCACAATGAGGGAA 660
P G R H

661 GGCATTGATGGGACCAGTTCTGCTGTGTATTCTCCTCCGTGCACAGTGAGGGAATCTATCAA 720

FIG. 5(CONTD.)

721 TAGTGCAATAA 731

60
1 GGCTTCGGCAGCCCACTCAGCGGTTTCGGCAGCCCACTCAGCGGTTTCGGCAGCCCACTC
G F G S P L S G F G S P L S G F G S P L

120
61 AGCGGCTTCGGCAGCCCACTCAGCGGATTTCGGTAGCCCACTCAGCGATTTCGGTAGCCCA
S G F G S P L S G F G S P L S G F G S P

180
121 CTCAGCGGATTTCGGTAGCCCACTTCGGCAGCTACGGTCCCCTGTCCATGGGTCTCGGAGCC
L S G F G S P F G S Y G P L S M G L G A

240
181 CCCAGGAGATTCCCCGGCGACCTCCGCCCTCATCTCTGAGCCCACTCCCGCCTTCCCGTT
P R R F P G D L R L I S E P T S R L P V

300
241 AGCGATGCCGTCTACACCGCTGTCTCCAGCCCGTCAAGCGCAGTGGTCCACACCGAG
S D A V Y T A V V Q P V T S A V V H T E

360
301 GGTCCCCATGTACCGGCCAAGTACAGGAACACGTTGCAATCTAAGCTTTCTAACCGCA
G P H V T G Q V Q E H V A I

420
361 AGCTATATTACGACGGATTAGTCAACACAGTCATCTTAAGCAAATGTATCTAAATAAAA

FIG. 6

421 TTTATCTGCCT 431

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FIG. 7

1	GGAGATCACCTGCTTGCAAAGGACAACGTCCTTAACACAGCCGCAAAATGAAAGCTTTCTT	60
	M K A F F	
61	CGTTCTTCCCTTCTTCAACCGCCGCACTGACGAATGCAGCAAGGGCTGGTCTGCTTGG	120
	V L S L L S T A A L T N A A R A G R L G	
121	AAGCGACCTGGATACATTTGGAAGGGTACACGGTAACCTATATGCCGGCATCGAAAGAGC	180
	S D L D T F G R V H G N L Y A G I E R A	
181	TGGCCCTCGTGGATACCCAGGGCTTACCGCATCGATTGGAGGCCGAAGTGGTGACGACT	240
	G P R G Y P G L T A S I G G E V G A R L	
241	CGGTGGTCCGGTGTGGAGTGAGCAGCTACGGCTATGTTACCCCTTCATGGGGCTA	300
	G G R A G V G V S S Y G Y G Y P S W G Y	
301	TCCGTATGGTGGATACGGTGGATACGGTGGATACGGTGGATATGATCAGGG	360
	P Y G G Y G G Y G G Y G G Y D Q G	

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361	TTTTGGCTCTGCATACGGCGGCTACCCCGCTACTATGGCTACTACTATCCAGTGGCTA	420
	F G S A Y G G Y P G Y Y G Y Y Y P S G Y	
421	CGGTGGGGGTACGGTGGTAGCTACGGTGGCAGCTACGGTGGTAGCTACACCTATCCCAA	480
	G G Y G G S Y G G S Y G G S Y T Y P N	
481	CGTTCGGGGCTTCAGCTGGTGCCGCGAGCTTGAGCTTCTCCTTCAGCGTCACAGTAAGAAAT	540
	V R A S A G A A A *	
541	CATGGAGCACCCGATCGAGAAATACAGAGGTTCTCAAAAGCGTACGGGATGCCAACCAGC	600
601	AAGAAATTGCGCCGCAAAATGTTGAGAACAAATACAAAGTTTCTGTAAANAAAAA	656

FIG. 7(CONTD.)

FIG. 8

1 ACGGACTAGGTTTCGCTGGCGTCCCTCTTATTGGCGGATACGGCTACGGTCTTTCGTTAG 60
G L G F A G V P L I G G Y G Y G P F V G

61 GAGCCTTCGCGTACGGCTTGTGGGTGGCCCTCGGTGGCTATGGCTACCCCTTCGGAC 120
A F A Y G L W G G L G G Y G Y P A F G L

121 TCTCCTGGGTTCCACATGGTTTGGAGGCTTGGAGCTTCTCCGCTGCTGCTGTTTCC 180
S W V P H G F G G F G A S P S A A G F R

181 GCTCGCTTGGAGCCTCTT 199
S L W S L

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FIG. 9

R.appendiculatus

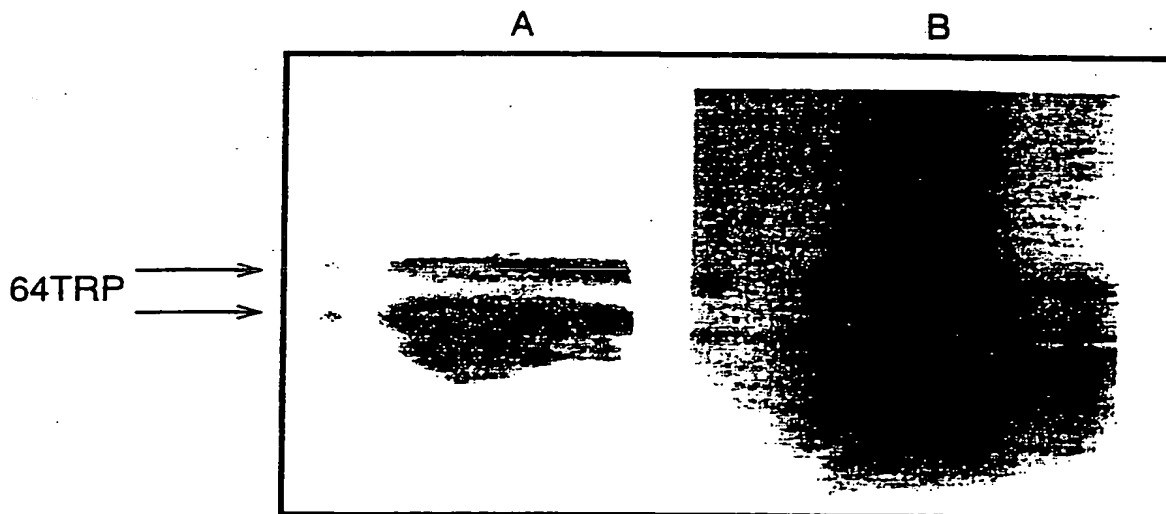
GLGFAGVPLIGGYGYGPFVGAFAAYGLWGLGGYGYPAFGLSWPHGFGFGASP
|.l: |.. :|l|l|l| :|:::|l : :|l|l|l| : | :::|l|l|l| :
GYGY.GAKKVGGYGYGAKLGGYGYG..AKIGGYGYGAKSGIQV.RALGGYGAGA

P.californica

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FIG. 10

EXPRESSION OF RECOMBINANT (TRUNCATED)
STRUCTURAL PROTEIN, 64TRP IN *Escherichia coli* CELLS



SDS-PAGE: (A) AND (B) WESTERN BLOT (USING ANTI-64TRP ANTISERUM) AND COOMASSIE BLUE STAINED 15% POLYACRYLAMIDE GEL, RESPECTIVELY OF IPTG-INDUCED *E.coli* CELLS EXPRESSING 64TRP PROTEIN. SAMPLES WERE SOLUBULISED AT 100°C IN SDS PRIOR TO LOADING THE GELS.

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FIG. 11a cdna inferred sequence alignment between the protein sequence of 64P and other related structural proteins.

ker2	T	E	L	R	R	T	V	Q	G	L	E	I	E	L	Q	S	L	A	L	K	Q	S	L	E	A	S	L	A	E	T	E	G	R	Y	C	V	Q	L	S	Q	I	Q	405		
ker3	T	E	L	R	R	T	V	Q	G	L	E	I	E	L	Q	S	L	A	L	K	Q	S	L	E	A	S	L	A	E	T	E	G	R	Y	C	V	Q	L	S	Q	I	Q	406		
ker1	E	V	V	K	K	Q	C	I	G	V	Q	D	S	I	A	D	A	E	Q	H	G	E	H	A	I	K	D	A	R	29	
ker4	S	E	L	N	R	V	I	Q	R	L	R	S	E	I	D	N	V	K	K	Q	I	S	N	L	Q	Q	S	I	S	D	A	E	Q	R	G	E	N	A	L	K	D	A	K	440	
64	0	
ker2	S	Q	I	S	A	L	E	E	Q	L	Q	Q	I	R	A	E	T	E	C	Q	N	A	E	Y	Q	Q	L	L	D	I	K	T	R	L	E	N	E	I	Q	T	Y	R	S	448	
ker3	S	Q	I	S	A	L	E	E	Q	L	Q	Q	I	R	A	E	T	E	C	Q	N	A	E	Y	Q	Q	L	L	D	I	K	T	R	L	E	N	E	I	Q	T	Y	R	S	449	
ker1	G	K	L	T	D	L	E	E	A	L	Q	Q	C	R	E	D	L	A	R	L	L	R	D	Y	Q	E	L	M	N	T	K	L	S	L	D	V	E	I	A	T	Y	R	K	72	
ker4	N	K	L	N	D	L	E	D	A	L	Q	Q	A	K	E	D	L	A	R	L	L	R	D	Y	Q	E	L	M	N	T	K	L	S	L	D	V	E	I	A	T	Y	R	T	483	
64	31	
ker2	L	L	E	G	E	G	S	S	S	G	G	466
ker3	L	L	E	G	E	G	S	S	S	G	G	467	
ker1	L	L	E	G	E	E	C	R	M	S	G	D	F	S	D	N	V	S	V	S	I	T	S	S	T	I	S	S	S	M	A	S	K	T	G	F	G	S	G	G	Q	S	115		
ker4	L	L	E	G	E	E	S	518	
64	36	
ker2	S	G	G	G	S	Y	G	G	S	S	G	G	S	Y	G	G	S	Y	G	G	S	S	G	G	S	Y	G	G	S	Y	G	G	S	Y	G	G	S	Y	G	G	S	506			
ker3	S	G	G	G	S	Y	G	G	S	S	G	G	S	Y	G	G	S	Y	G	G	S	S	G	G	S	Y	G	G	S	Y	G	G	S	Y	G	G	S	Y	G	G	S	507			
ker1	G	G	R	G	S	Y	G	G	R	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	157		
ker4	G	G	G	G	G	Y	553		
64	N	L	Y	A	G	I	E	R	A	G	P	R	G	Y	P	G	L	T	A	S	I	G	G	E	V	G	A	R	L	G	G	R	A	G	V	G	S	S	Y	G	Y	78			

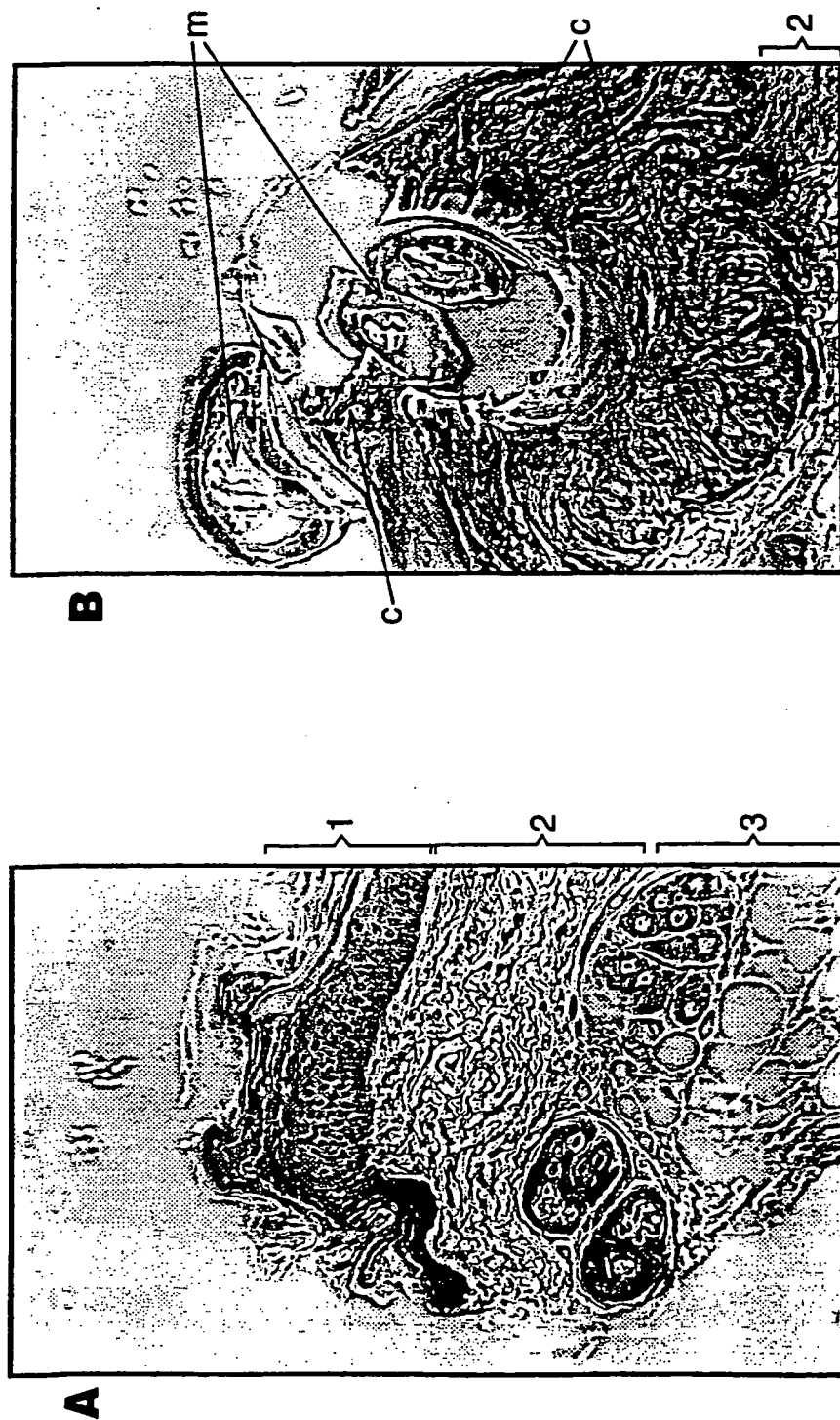
Sequences are: ker1=keratin complex2 basic protein, ker2=mouse keratin, ker3=mouse epidermalK protein, ker4=human keratin protein, 64P=R. appendiculatus putative structural protein

	col1	col2	col3	col4	col5	col6	col7	col8	col9	col10	col11	col12	col13	col14	col15	col16	col17	col18	col19	col20	col21	col22	col23	col24	col25	col26	col27	col28	col29	col30	col31	col32	col33	col34	col35	col36	col37	col38	col39	col40	col41	col42	col43	col44	col45	col46	col47	col48	col49	col50	col51	col52	col53	col54	col55	col56	col57	col58	col59	col60	col61	col62	col63	col64	col65	col66	col67	col68	col69	col70	col71	col72	col73	col74	col75	col76	col77	col78	col79	col80	col81	col82	col83	col84	col85	col86	col87	col88	col89	col90	col91	col92	col93	col94	col95	col96	col97	col98	col99	col100	col101	col102	col103	col104	col105	col106	col107	col108	col109	col110	col111	col112	col113	col114	col115	col116	col117	col118	col119	col120	col121	col122	col123	col124	col125	col126	col127	col128	col129	col130	col131	col132	col133	col134	col135	col136	col137	col138	col139	col140	col141	col142	col143	col144	col145	col146	col147	col148	col149	col150	col151	col152	col153	col154	col155	col156	col157	col158	col159	col160	col161	col162	col163	col164	col165	col166	col167	col168	col169	col170	col171	col172	col173	col174	col175	col176	col177	col178	col179	col180	col181	col182	col183	col184	col185	col186	col187	col188	col189	col190	col191	col192	col193	col194	col195	col196	col197	col198	col199	col200	col201	col202	col203	col204	col205	col206	col207	col208	col209	col210	col211	col212	col213	col214	col215	col216	col217	col218	col219	col220	col221	col222	col223	col224	col225	col226	col227	col228	col229	col230	col231	col232	col233	col234	col235	col236	col237	col238	col239	col240	col241	col242	col243	col244	col245	col246	col247	col248	col249	col250	col251	col252	col253	col254	col255	col256	col257	col258	col259	col260	col261	col262	col263	col264	col265	col266	col267	col268	col269	col270	col271	col272	col273	col274	col275	col276	col277	col278	col279	col280	col281	col282	col283	col284	col285	col286	col287	col288	col289	col290	col291	col292	col293	col294	col295	col296	col297	col298	col299	col300	col301	col302	col303	col304	col305	col306	col307	col308	col309	col310	col311	col312	col313	col314	col315	col316	col317	col318	col319	col320	col321	col322	col323	col324	col325	col326	col327	col328	col329	col330	col331	col332	col333	col334	col335	col336	col337	col338	col339	col340	col341	col342	col343	col344	col345	col346	col347	col348	col349	col350	col351	col352	col353	col354	col355	col356	col357	col358	col359	col360	col361	col362	col363	col364	col365	col366	col367	col368	col369	col370	col371	col372	col373	col374	col375	col376	col377	col378	col379	col380	col381	col382	col383	col384	col385	col386	col387	col388	col389	col390	col391	col392	col393	col394	col395	col396	col397	col398	col399	col400	col401	col402	col403	col404	col405	col406	col407	col408	col409	col410	col411	col412	col413	col414	col415	col416	col417	col418	col419</
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Sequences are: col1=Glycine-rich GX protein-motif LGG, col2=collagen type Iv alpha protein, col3=IPB2 precursor protein, col4=Glycine rich cell wall protein, 64TRP=R. appendiculatus truncated structural protein

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FIG. 12a HISTOLOGICAL STUDIES OF THIN HAMSTER SKIN SECTIONS STAINED WITH HAEMATOXYLIN AND EOSIN

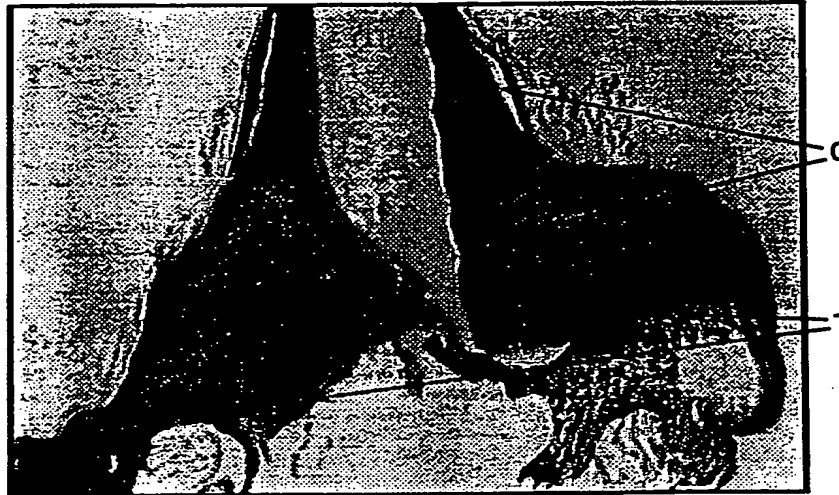
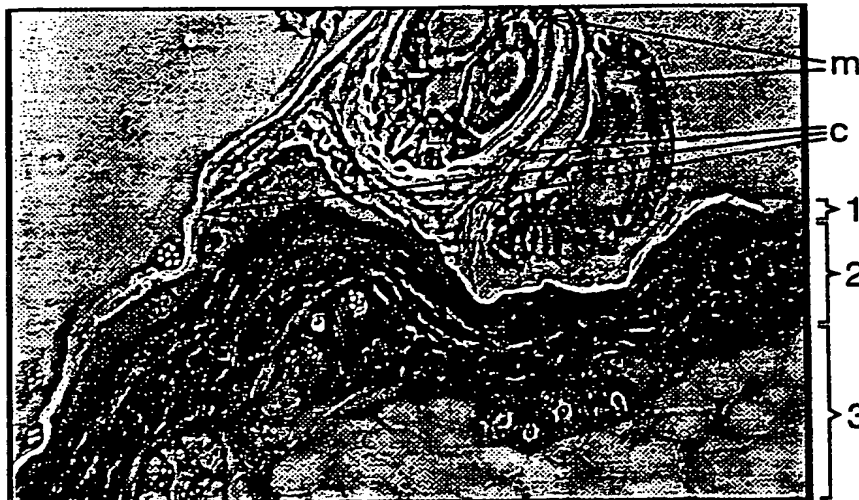


(A) AND (B) = HISTOLOGICAL SECTIONS OF HAMSTER SKIN STAINED WITH HAEMATOXYLIN AND EOSIN;
 (A)=NORMAL SKIN SECTION SHOWING EPIDERMIS-1, DERMIS-2 AND SUBCUTANEOUS LAYER-3.
 (B)=SKIN SECTION SHOWING AN EMBEDDED TICK (*R. appendiculatus*) CEMENT CONE. STAINING: PINK=
 BASEMENT MEMBRANES/COLLAGEN FIBRES, UNSTAINED=RETICULIN FIBRES, BLUE=CELL
 NUCLEI. C REFERS TO CEMENT CONE AND M TICK MOUTH PARTS. ABOUT 20x

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FIG. 12b

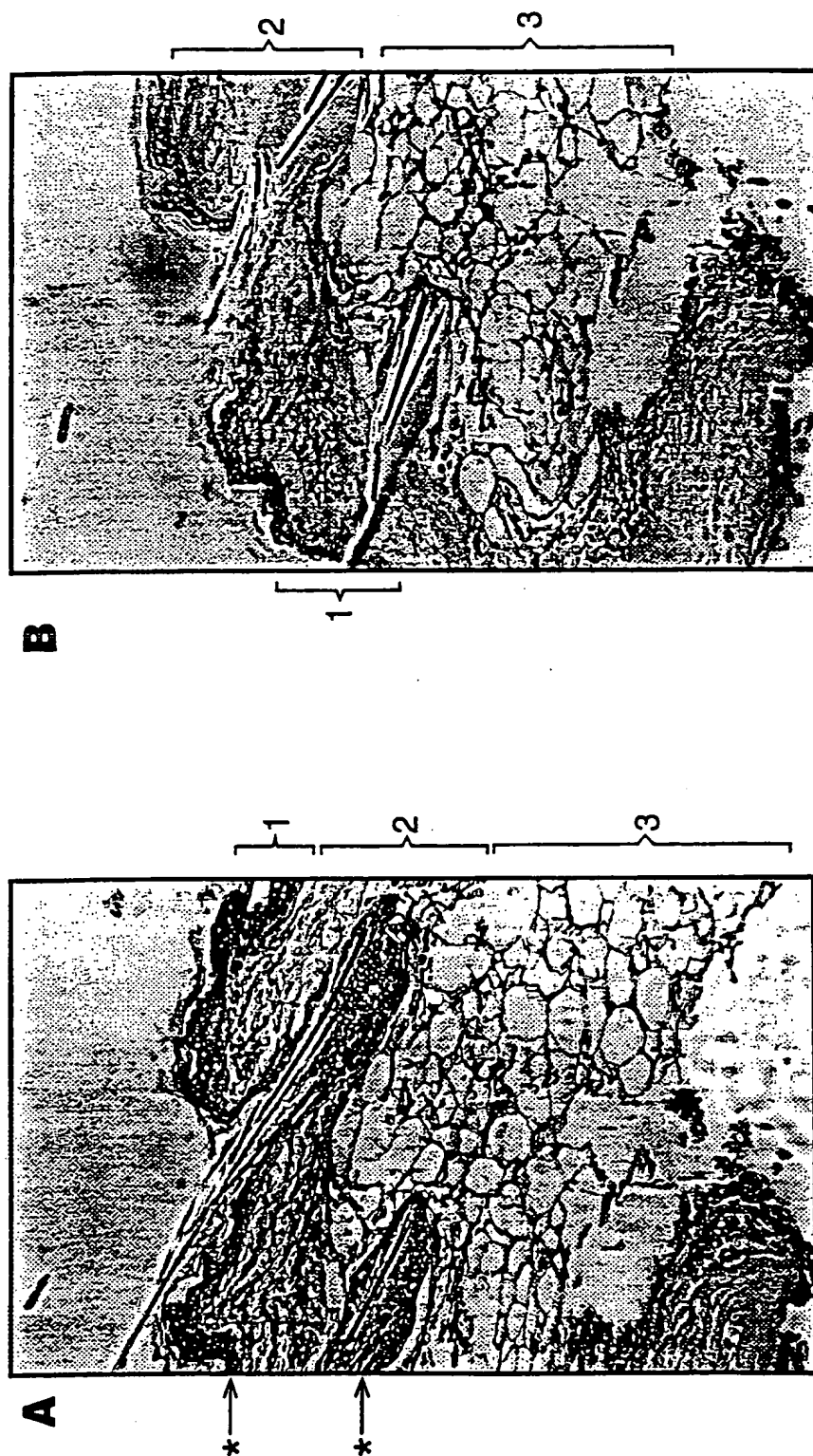
HISTOLOGICAL STUDIES OF THIN HAMSTER SKIN SECTIONS
STAINED WITH HAEMATOXYLIN AND EOSIN, AND VAN GIESON STAINS.

A**B**

(A) AND (B) = HISTOLOGICAL SECTIONS OF HAMSTER SKIN SHOWING ATTACHED TICK (*R. appendiculatis*) CEMENT CONES (i.e. POST FEEDING) STAINED WITH (A) HAEMATOXYLIN AND EOSIN STAIN (H & E), (B) VAN GIESON STAIN. 1=EPIDERMIS, 2=DERMIS AND 3=SUBCUTANEOUS LAYER. STAINS: H & E-PINK=BASEMENT MEMBRANES/COLLAGEN FIBRES, BLUE=CELL NUCLEI, VAN GEISON-RED=COLLAGEN FIBRES, YELLOW=BASEMENT MEMBRANES/RETICULIN AND ELASTIN FIBRES. C=CEMENT CONE AND M= TICK MOUTHPARTS. ABOUT 20x.

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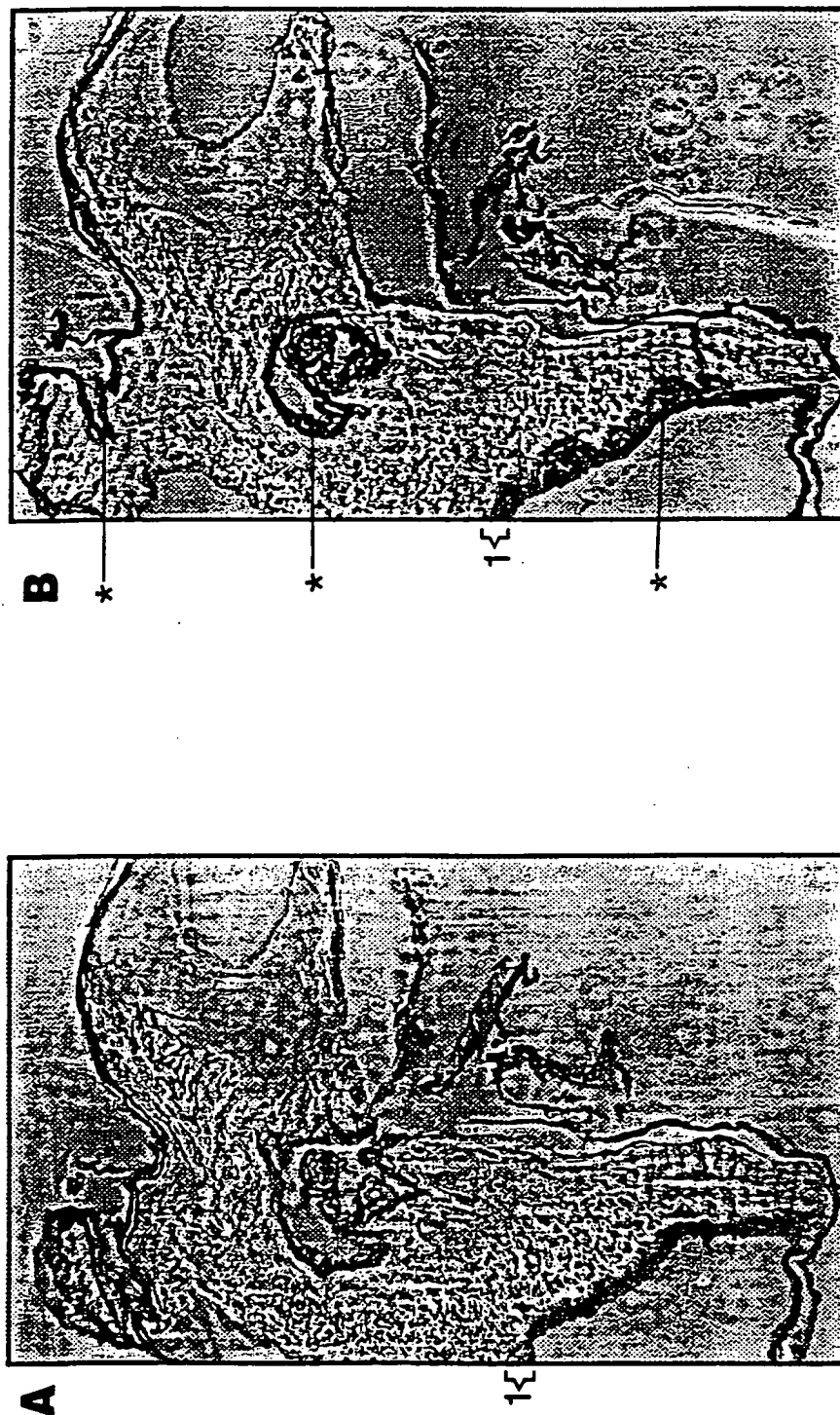
FIG. 13a IMMUNOPEROXIDASE STUDIES ON HAMSTER
SKIN SECTIONS USING ANTI-64TRP ANTISERUM



(A) AND (B) = NORMAL HISTOLOGICAL SECTIONS OF HAMSTER SKIN INCUBATED WITH ANTI-64TRP ANTISERUM AND A POSITIVE CONTROL ANTISERUM RESPECTIVELY, AND DEVELOPED WITH PEROXIDASE LINKED SECONDARY ANTISERUM. 1= EPIDERMIS, 2=DERMIS AND 3= SUBCUTANEOUS LAYER; *=-AREAS OF SKIN SHOWING POSITIVE REACTIONS TO ANTI 64TRP ANTISERUM. ABOUT 20x

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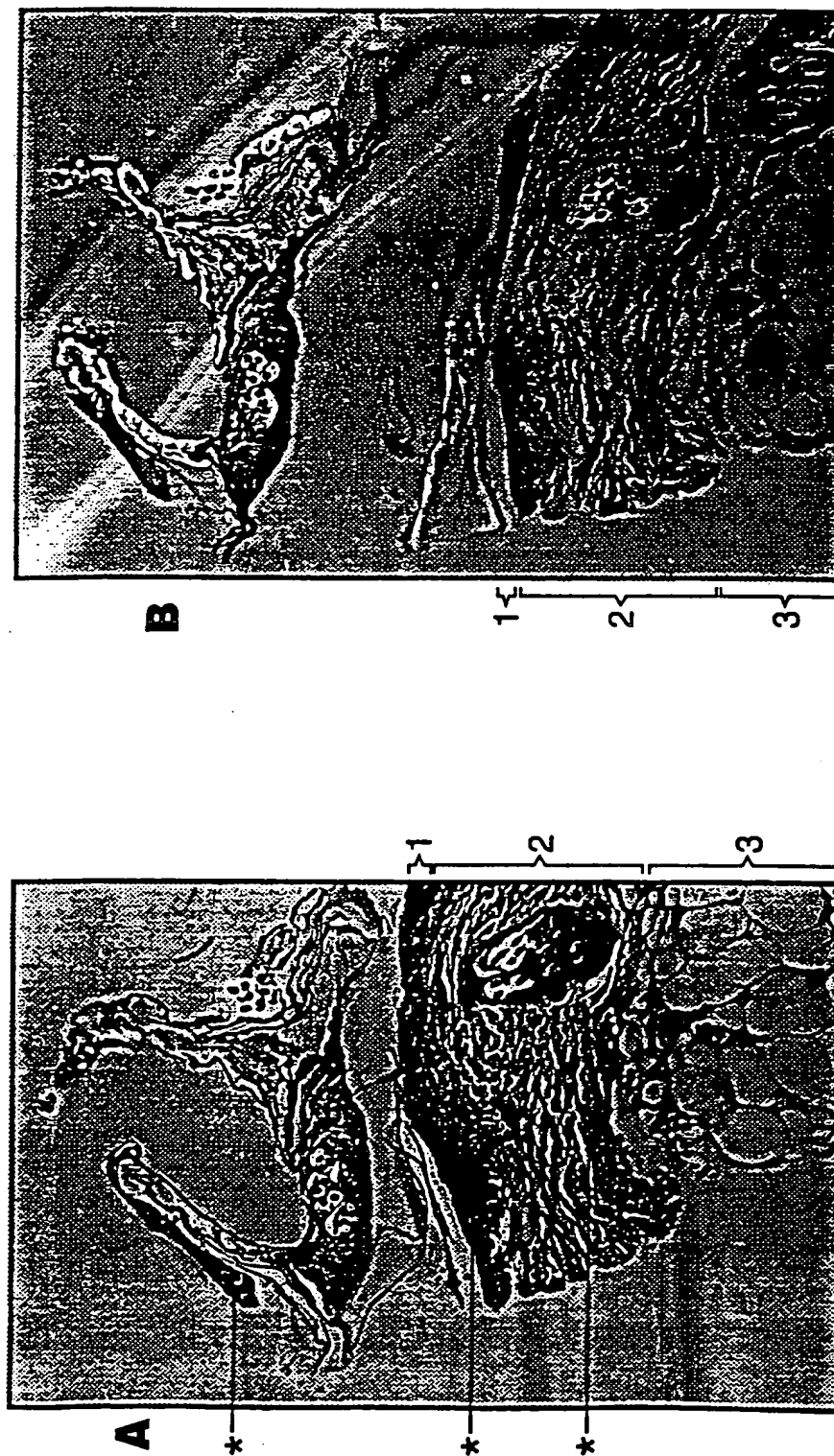
FIG. 13b IMMUNOPEROXIDASE STUDIES ON *R. appendiculatus* CEMENT
CONE SECTIONS USING ANTI-64TRP ANTISERUM



(A) AND (B) = THIN SECTIONS OF *R. appendiculatus* CEMENT CONES INCUBATED WITH EITHER A POSITIVE CONTROL ANTI-SERUM OR ANTI-64 TRP ANTI-SERUM RESPECTIVELY, AND DEVELOPED WITH PEROXIDASE LINKED SECONDARY ANTISERUM. 1=PART OF SKIN EPIDERMIS
*=AREAS OF SKIN SHOWING POSITIVE REACTIONS TO ANTI-64TRP ANTI-SERUM. ABOUT 20x

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FIG. 13c IMMUNOPEROXIDASE STUDIES USING ANTI-64TRP ANTISERUM ON THIN HAMSTER SKIN SECTIONS POST FEEDING WITH *R. appendiculatus*



(A) AND (B) = THIN SECTIONS OF HAMSTER SKIN POST-FEEDING WITH *R. appendiculatus* SHOWING A CEMENT CONE STILL ATTACHED, INCUBATED WITH ANTI-64TRP ANTISERUM AND A POSITIVE CONTROL ANTISERUM RESPECTIVELY, AND DEVELOPED WITH PEROXIDASE LINKED SECONDARY ANTISERUM. 1=EPIDERMIS, 2=DERMIS AND 3=SUBCUTANEOUS LAYER. * = AREAS OF SKIN SHOWING POSITIVE REACTIONS TO ANTI 64TRP ANTISERUM. ABOUT 20x